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REPORT

MRL-R-794

A COMPARISON OF THE ANTIMUSCARINIC PROPERTIES OF APROPHEN WITH THOSE OF SOME OTHER ANTICHOLINERGIC DRUGS

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Concentrations of aprophen in the millimolar range were necessary for it to have any effect on the kinetics of acetylcholinesterase. The effects of aprophen on the enzyme were similar to those previously reported for benactyzine (Dawson and Bladen 1979).

The results extend and complement those of Green et. al. (1980) who showed that aprophen was as effective as atropine in the treatment of nerve agent poisoning.

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16. *ABSTRACT (if this is security classified, the announcement of this report will be similarly classified):*

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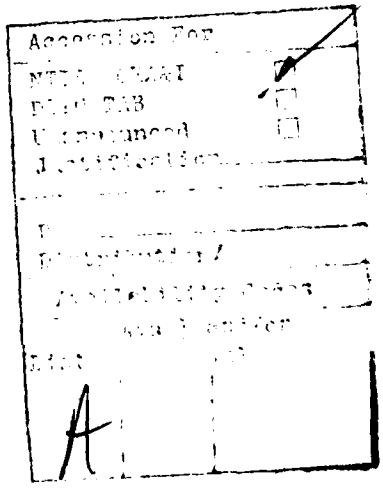
The results extend and complement those of Green et. al. (1980) who showed that aprophen was as effective as atropine in the treatment of nerve agent poisoning.



CONTENTS

A COMPARISON OF THE ANTIMUSCARINIC PROPERTIES OF APROPHEN WITH THOSE OF SOME OTHER ANTICHOLINERGIC DRUGS

1. INTRODUCTION

There has been interest over a period of years in the possibility of finding an anticholinergic drug which might prove superior to atropine in the treatment of nerve agent poisoning. One could search for a more potent anticholinergic, one with an increased duration of action in the body, or one with better access to the central nervous system. Recent work with benactyzine and the related drug aprophen (in which the OH group of benactyzine has been replaced by CH_3) suggests further that less specific antimuscarinic drugs may be more effective antidotes than atropine (Green et al., 1980). Whether this efficacy derives from blockade of receptors other than the muscarinic cholinoceptor is uncertain, but the subject is of sufficient importance to warrant further pharmacological study.

Green et al. (1980) evaluated the antimuscarinic properties of atropine and aprophen in the peripheral and central nervous systems by antagonism of carbachol-induced contractions of the guinea pig ileum, and antagonism of oxotremorine-induced tremors in mice, respectively. In the present work we have determined apparent dissociation constants for a number of antimuscarinic drugs (including aprophen) in the guinea pig atrium by the method of Arunlakshana and Schild (1959). In addition, dissociation constants for atropine, aprophen and benactyzine in guinea pig brain and ileum were determined by direct binding studies using the radiolabelled antagonist (^{3}H)-quinuclidinyl-benzilate (QNB). Interactions between aprophen and the enzyme acetylcholinesterase (AChE; EC 3.1.1.7) were also studied.

The results indicate that aprophen and benactyzine are of comparable potency, and both are weaker antagonists of the muscarinic receptor than atropine. It was also noted that the muscarinic receptor shows some degree of regional heterogeneity; anticholinergic drugs bind more tightly in the central nervous system than in the periphery.

2. EXPERIMENTAL PROCEDURES

2.1 Cardiac Muscarinic Receptors

The apparent dissociation constant of a number of antagonist drugs was determined for the muscarinic receptor of the guinea pig atrium. Experiments were carried out at 37°C, and acetylcholine chloride was used as the agonist. Log-log plots of dose ratio minus one (DR-1) versus antagonist concentration were constructed (Arunlakshana and Schild, 1959) and the apparent dissociation constant, K_i, determined from the intercept at (DR-1) = 1 by means of a least squares regression.

2.2 Direct Binding Studies

Homogenates of guinea pig brain and ileum were prepared and assayed for muscarinic receptors with (^{3}H)-QNB as described by Dawson and Jarrott (1980). Inhibition of QNB binding (44-74 pM) by atropine, benactyzine or aprophen was analysed by means of a Hill plot (equation 1) where Y is the ratio of QNB bound in the presence of inhibitor of concentration I to that in its absence and n_H is the Hill coefficient (Fields et al., 1978).

$$\log [Y/(1-Y)] = n_H \log I \qquad (1)$$

Apparent values of K_i, the dissociation constant of the inhibitor-receptor complex, were calculated using equation 2 where I_{50} is the value of I corresponding to Y = 0.5, K_D is the apparent dissociation constant of the QNB-receptor complex and Q is the concentration of QNB. Values of K_D were taken from Dawson and Jarrott (unpublished results).

$$K_i = I_{50}/(1 + Q/K_D) \qquad (2)$$

2.3 Enzyme Studies

Bovine erythrocyte AChE (Sigma) was used. Hydrolysis of the substrate acetylthiocholine, decarbamylation of dimethylcarbamyl-AChE (both in 45 mM phosphate at 25°C), dephosphorylation of diethylphosphoryl-AChE (in 2.5 mM phosphate - 150 mM NaCl at 37°) and ageing of isopropylmethylphosphonyl-AChE (in 2 mM phosphate - 10 mM NaCl at 37°) were studied as described by Dawson and Bladen (1979).

3. RESULTS AND DISCUSSION

3.1 Muscarinic Cholinoceptors

Atropine, benactyzine and aprophen all inhibited binding of the antagonist (^{3}H)-QNB to muscarinic receptors of brain and ileum of the guinea pig with a Hill coefficient, n_H, which was statistically indistinguishable from 1.0 in all cases (Table 1). This indicates a lack of cooperativity in binding and is

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characteristic of muscarinic antagonists (Hulme et al., 1978). The three drugs were all potent inhibitors of QNB binding, with K_i values in the range 0.6-9 nM (Table 1). Table 1 also lists apparent values of K_i for a number of muscarinic antagonists acting on the guinea pig atrium. In the two brain regions studied (striatum and pons-medulla) and in the ileum, atropine had the lowest K_i value and benactyzine the highest, although there was little difference between benactyzine and aprophen. These results are consistent with those of Green et al. (1980) who measured antimuscarinic potency by other methods (see Introduction). In the atrium the R-enantiomer of the glycollate T3436 was the most potent antagonist and aprophen was the least potent. The value for K_i for atropine in the atrium is identical with that found in the ileum. The K_i values for aprophen and benactyzine are also similar in atrium and ileum, but the order of potency is reversed. Aprophen is slightly less potent than benactyzine in the atrium, and slightly more potent in the ileum. The differences may be more a function of the assay procedures than of any physiological importance.

It is interesting to note that for any one drug, K_i varied significantly from striatum to pons-medulla to ileum (with the exception that the difference between K_i for benactyzine/ileum and K_i for benactyzine/pons-medulla was not statistically significant). Such regional heterogeneity is consistent with observations on QNB (Dawson and Jarrott, unpublished results) and other antagonists acting on brain muscarinic receptors (Kloog et al., 1979). This heterogeneity may well be of physiological importance. One may speculate that postsynaptic receptors may have a similar configuration, but differ from presynaptic receptors. Regional heterogeneity could then reflect differences in the distribution of presynaptic and postsynaptic receptors.

3.2 Interactions of Aprophen with AChE

Aprophen was found to be a mixed competitive-uncompetitive inhibitor of AChE. The competitive and uncompetitive dissociation constants were found to be 0.27 ± 0.32 mM and 2.9 mM (range, 0.9 - 4.6 mM) respectively. These figures are similar to those for benactyzine, 0.63 mM and 20.7 mM respectively (Dawson and Bladen, 1979). Aprophen is a slightly more potent inhibitor than benactyzine, but both are comparatively weak inhibitors.

Aprophen at the relatively high concentration of 1 mM had negligible effects on the rates of ageing of isopropylmethylphosphonyl-AChE and dephosphorylation of diethylphosphoryl-AChE, but inhibited the rate of decarbamylation of dimethylcarbamyl-AChE (Table 2) by 32%. Corresponding effects have been observed for benactyzine (Dawson and Bladen, 1979). The dissociation constant for the benactyzine-dimethylcarbamyl-AChE complex was calculated as 2.9 mM (Dawson and Bladen, 1979). Benactyzine and aprophen are therefore approximately equi-effective with respect to decarbamylation.

Overall, the effects of aprophen on AChE are of no significance in assessing the therapeutic actions of aprophen *in vivo*.

4. ACKNOWLEDGEMENTS

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We are grateful to Chemical Defence Establishment, Porton for their gifts of aprophen and T3436.

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TABLE 1

APPARENT DISSOCIATION CONSTANTS AND HILL COEFFICIENTS OF ANTAGONISTS OF MUSCARINIC CHOLINOCEPTORS OF GUINEA PIG BRAIN, ILEUM AND ATRIUM

Parameter	Region			
	Ileum	Pons-medulla	Striatum	Atrium
K_i (nM)				
Atropine	2.15 ± 0.15	1.02 ± 0.10	0.56 ± 0.05	2.2
Aprophen	8.00 ± 1.68	3.75 ± 0.23	2.29 ± 0.32	10.3
Benactyzine	9.35 ± 0.85	7.10 ± 0.45	3.42 ± 0.61	7.8
Hyoscine				0.9
T3436 (racemate)				0.9
T3436 (R)				0.3
n_H				
Atropine	0.95 ± 0.05	1.06 ± 0.05	1.03 ± 0.06	
Aprophen	1.01 ± 0.04	1.26 ± 0.09	1.08 ± 0.03	
Benactyzine	1.08 ± 0.08	1.05 ± 0.02	0.96 ± 0.06	

n_H is defined in equation 1.
For direct binding studies, concentration of (^{3}H)-QNB = 44-74 pM.
Results for brain regions and ileum are given as the mean and standard error from four animals.

TABLE 2

EFFECT OF APROPHEN ON RATE CONSTANTS FOR REACTIVATION AND AGEING OF INHIBITED ACETYLCHOLINESTERASE

	Rate constant k (hr^{-1})		
	Dephosphorylation	Decarbamylation	Ageing
Control	0.0147 ± 0.0010 (3)	0.344 ± 0.014 (4)	0.315 ± 0.009 (2)
1 mM aprophen	0.0162 ± 0.0008 (2)	0.235 ± 0.013 (5)*	0.288 ± 0.030 (2)

Inhibited AChE = diethylphosphoryl-AChE (for dephosphorylation)
dimethylcarbamyl-AChE (for decarbamylation)
isopropylmethylphosphonyl-AChE (for ageing)

*Significantly different from control; $P < 0.05$

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